VARIATION IN THE EXPRESSION OF THE RAGGED MUTANT IN NEUROSPORA

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THE mutant ragged (*rg*) in *Neurospora crassa* has been described by PERKINS (1959). Later, this gene was introduced into *N. sitophila* by THRELKELD (1961, 1962a, b). Linkage data from random spore analysis in *N. crassa* (DESERRES 1958; PERKINS 1959) and from tetrad analysis in *N. sitophila* (THRELKELD 1961, 1962a, b) show that *rg* is very close to the centromere on linkage group I, whether right or left is not known. During the present investigation in *N. sitophila* some progeny with *rg* phenotype from the cross *rg* × wild type, were found to give the wild-type phenotype in a heterokaryon between two *rg* homokaryons. The *rg* isolates also produce wild-type progeny in crosses of *rg* × *rg*. The present investigation includes a detailed genetic analysis of these phenomena.

MATERIALS AND METHODS

Neurospora sitophila wild-type strains were obtained from DR. H. L. K. WHITEHOUSE, these have been described earlier by RAMSBOTTOM and STEPHENS (1935). The *N. sitophila* strain designated as NSa is phenotypically wild type, and was recovered from a series of backcrosses following an initial hybridization of *N. sitophila* × *N. crassa* as derived in an earlier study (THRELKELD 1961). Other *N. sitophila* wild-type strains used were FGSC Nos. 346, 412, 414, 415, 417; these were obtained from the Fungal Genetics Stock Center (FGSC), Dartmouth College, Hanover, New Hampshire. In *N. crassa*, the St. Lawrence wild-type strains 74A and 74-OR8-1a, obtained from FGSC, were used; these have been described by CASE, BROCKMAN and DESERRES (1965).

The *N. crassa rg* mutant B53 induced in 74A background by DR. VAL WOODWARD (PERKINS 1959) was kindly donated by DR. PERKINS. The *rg* marker was introduced into *N. sitophila* by several backcrosses of the original *N. crassa rg* B53 with *N. sitophila* Whitehouse wild-type strains. The *cr* (crisp, linkage group I) and *ylo* (yellow, linkage group VI) markers were also introduced into *N. sitophila* by several backcrosses of the original *N. crassa cr* and *ylo* mutants with *N. sitophila* Whitehouse wild-type strains by THRELKELD (1961).

The strains were maintained on WESTERGAARD and MITCHELL (1947) minimal medium. Mutant strains could easily be distinguished from wild type after two days growth (Figure 1). All crosses were made on malt peptone agar medium (THRELKELD 1962a) by inoculating conidia of the two mating types simultaneously. Standard methods were used for both random spore analysis and tetrad analysis (CATCHESIDE 1951). Conidia were harvested in sterile distilled water and filtered through glass wool. Conidial isolates were obtained by plating conidia on minimal medium containing sorbose at 4 mg/ml and sucrose at 2 mg/ml. Heterokaryon tests were done in pairwise combinations of mutant isolates on minimal agar slants.

RESULTS

The two *rg* isolates of the pairwise combinations which, in a heterokaryon test

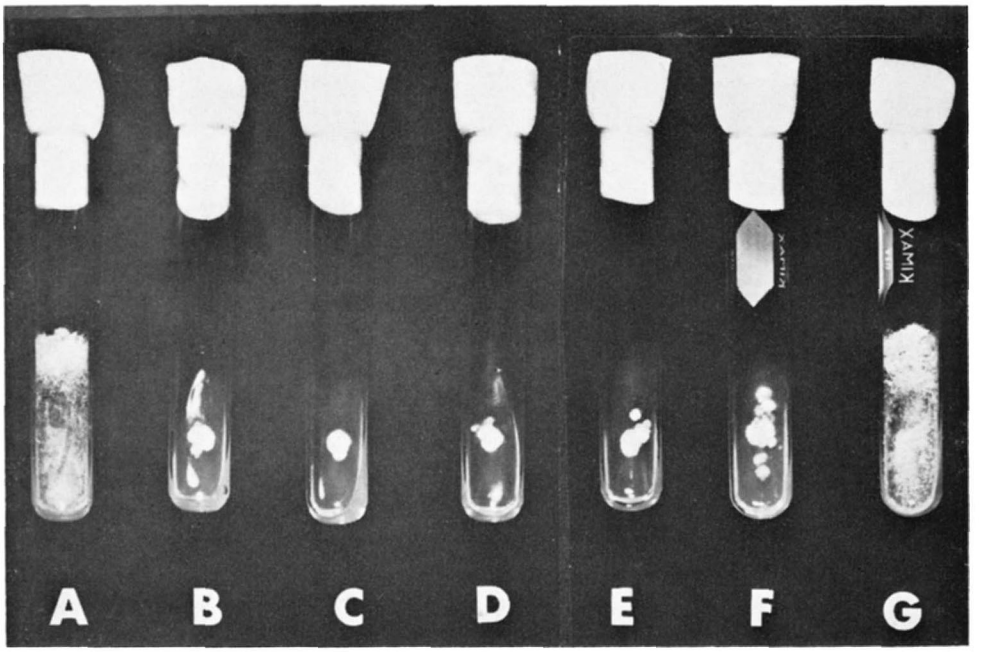

FIGURE 1.—Growth forms of the different *N. sitophila* strains on 2% glucose minimal agar. A. wild type. B, C, D. mutant isolates having ragged phenotype. E, F. pairwise combination of *rg type 0* + *rg type-1* (E) and *rg type-0* + *rg type-2* (F); both showing mutant phenotype in heterokaryons. G. combination of *rg type-1* + *rg type-2* showing wild-type growth in heterokaryon.

resulted in the wild-type phenotype, were arbitrarily called *rg type-1* and *rg type-2*, while those *rg* isolates which failed to give the wild-type phenotype in pairwise combination with either of these two complementing types were called *rg type-0*. The complementing types of *rg* isolates could easily be distinguished from the noncomplementing type of *rg* isolates after three days growth of the heterokaryon on minimal agar (Figure 1). The fact that *rg type-1*, *rg type-2* and *rg type-0* were heterokaryon compatible with each other, was determined by their ability to show wild type growth and pink color in pairwise combination with a tester isolate having *cr;ylo* phenotype. Evidence for heterokaryon formation between *rg type-1* and *rg type-2* isolates, was obtained in the following way. A minimal glucose agar slant was inoculated with conidia from *rg type-1* and *rg type-2* isolates simultaneously. After five days growth, conidial isolates were obtained. Conidia from the wild-type colony so obtained were found on subsequent analysis to give rise to *rg* colonies. Further evidence for heterokaryon formation was furnished from the recovery of *rg* progeny from a cross between two heterokaryons obtained as single wild-type conidial isolates. The progeny with the wild-type phenotype obtained from the cross *rg type-1* × *rg type-2* have been designated "apparent wild type" (AWT). Information regarding such isolates characterised during the present study is given in Table 1.

The genotype of the noncomplementing *rg* (*rg type-0*) was confirmed by the

TABLE 1

Phenotype and inferred genotype of N. sitophila *and* N. crassa *strains, with regard to* rg-1, rg-2 *and* su-2 *genes*

Species	Strains	Mating type	*Phenotype	Genotype		
N. sitophila	Wa (Whitehouse) FGSC No. 412	a	wild type	*rg-1*+	*rg-2*+	*su-2*+
	WA (Whitehouse) FGSC No. 346, 414, 415, 417	A				
	NSa, 15–6	a	Apparent			
	15–7	A	wild type	*rg-1*+	*rg-2*	*su-2*
	M–13, M–16	a	*rg type-0*	*rg-1*	*rg-2*	*su-2*+
	M–17	A	*rg type-1*	*rg-1*	*rg-2*+	*su-2*+
	SFT–1	A	*rg type-1*	*rg-1*	*rg-2*+	*su-2*
	B–1	a				
	SFT–2	a	*rg type-2*	*rg-1*+	*rg-2*	*su-2*+
	SFT–5, SFT–9	A				
N. crassa	74A	A	wild type	*rg-1*+	*rg-2*+	*su-2*+
	74–OR8–1a	a				
	B53	A	ragged	*rg-1*	*rg-2*+	*su-2*+

* The phenotype of the mutant isolates was determined by heterokaryon tests with known *rg type-1* (SFT–1), *rg type-2* (SFT–9) and *cr*; *ylo* (No. 50-2) tester isolates.

failure to recover wild-type (wt) progeny from crosses between *rg type-0* with a known *rg type-1* strain (1000 spores germinated) and with a known *rg type-2* strain (100 spores germinated). No wild-type progeny were recovered from a cross between *rg type-1* and *rg type-1* (5000 spores germinated) in *N. sitophila.*

The results of the various crosses are summarized in Table 2 (data from random spore analysis) and Table 3 (data from tetrad analysis).

Crosses *rg type-1* × *rg type-2*, *rg type-1* (M-17) × NSa and *rg type-1* (M-17) × AWT (15–6) produced *rg* and wild-type progeny in a ratio not differing significantly from 5:3. On further analysis, the *rg* progeny from these crosses were found to show the three *rg* phenotypes *rg type-0*, *rg type-1* and *rg type-2*. Tetrad analysis of these last three crosses showed three different classes of asci:

Class I	4*rg*:4wt	In some asci, the mutant progeny were all *rg type-0* while in others the mutant progeny were all *rg type-1*.
Class II	8*rg*:0wt	Among the progeny *rg type-1* and *rg type-2* occurred in equal numbers.
Class III	6*rg*:2wt	Asci were found to be of two types: (a) among the mutant progeny the three *rg* phenotypes occurred in equal numbers. (b) among the mutant progeny *rg type-1* and *rg type-2* occurred in a ratio of 2:1.

Crosses *rg type-1* (SFT-1) × NSa and *rg type-1* (SFT-1) × 15–6 (AWT) produced *rg* and wild-type progeny in the ratio of 1:1; tetrad analysis from these crosses produced only one class of asci having 4*rg* and 4wt in each ascus; the mutant progeny from these crosses were *rg type-1*. Crosses *rg type-2* (SFT-5) ×

TABLE 2

Genetic analysis of random isolates from crosses involving rg-1, rg-2, su-2 *genes in* N. sitophila

Cross	Parent types and inferred genotypes	Ratio ragged: wild type in the progeny	Phenotype of the mutant progeny
B–1 × SFT–9	*rg type-1* (*rg-1 rg-2+ su-2*) × *rg type-2* (*rg-1+ rg-2 su-2+*)	5:3	*rg type-0, rg type-1, rg type-2*
M–17 × NSa	*rg type-1* (*rg-1 rg-2+ su-2+*) × Apparent wild type (*rg-1+ rg-2 su-2*)	5:3	*rg type-0, rg type-1, rg type-2*
M–17 × 15–6	*rg type-1* (*rg-1 rg-2+ su-2+*) × Apparent wild type (*rg-1+ rg-2 su-2*)	5:3	*rg type-0, rg type-1, rg type-2*
M–17 × Wa	*rg type-1* (*rg-1 rg-2+ su-2+*) × wild type (*rg-1+ rg-2+ su-2+*)	1:1	*rg type-1*
M–17 × 412	*rg type-1* (*rg-1 rg-2+ su-2+*) × wild type (*rg-1+ rg-2+ su-2+*)	1:1	*rg type-1*
M–17 × SFT–2	*rg type-1* (*rg-1 rg-2+ su-2+*) × *rg type-2* (*rg-1+ rg-2 su-2+*)	3:1	.....
M–13 × WA	*rg type-0* (*rg-1 rg-2 su-2+*) × wild type (*rg-1+ rg-2+ su-2+*)	3:1	*rg type-0, rg type-1, rg type-2*
M–16 × WA	*rg type-0* (*rg-1 rg-2 su-2+*) × wild type (*rg-1+ rg-2+ su-2+*)	3:1	*rg type-0, rg type-1, rg type-2*
M–16 × 346	*rg type-0* (*rg-1 rg-2 su-2+*) × wild type (*rg-1+ rg-2+ su-2+*)	3:1	*rg type-0, rg type-1, rg type-2*
M–16 × 414	*rg type-0* (*rg-1 rg-2 su-2+*) × wild type (*rg-1+ rg-2+ su-2+*)	3:1	*rg type-0, rg type-1, rg type-2*
M–16 × 415	*rg type-0* (*rg-1 rg-2 su-2+*) × wild type (*rg-1+ rg-2+ su-2+*)	3:1	*rg type-0, rg type-1, rg type-2*

TABLE 2—Continued

Cross	Parent types and inferred genotypes		Ratio ragged: wild type in the progeny	Phenotype of the mutant progeny
M–16 × 417	*rg type-0* (*rg-1 rg-2 su-2*+)	× wild type (*rg-1*+ *rg-2*+ *su-2*+)	3:1	*rg type-0*, *rg type-1*, *rg type-2*
SFT–1 × NSa	*rg type-1* (*rg-1 rg-2*+ *su-2*)	× Apparent wild type (*rg-1*+ *rg-2 su-2*)	1:1	*rg type-1*
SFT–1 × 15–6	*rg type-1* (*rg-1 rg-2*+ *su-2*)	× Apparent wild type (*rg-1*+ *rg-2 su-2*)	1:1	*rg type-1*
SFT–1 × Wa	*rg type-1* (*rg-1 rg-2*+ *su-2*)	× wild type (*rg-1*+ *rg-2*+ *su-2*+)	1:1	*rg type-1*
SFT–5 × NSa	*rg type-2* (*rg-1*+ *rg-2 su-2*+)	× Apparent wild type (*rg-1*+ *rg-2 su-2*)	1:1	*rg type-2*
SFT–5 × 15–6	*rg type-2* (*rg-1*+ *rg-2 su-2*+)	× Apparent wild type (*rg-1*+ *rg-2 su-2*)	1:1	*rg type-2*
SFT–5 × Wa	*rg type-2* (*rg-1*+ *rg-2 su-2*+)	× wild type (*rg-1*+ *rg-2*+ *su-2*+)	1:1	*rg type-2*
15–7 × 412	Apparent wild type (*rg-1*+ *rg-2 su-2*)	× wild type (*rg-1*+ *rg-2*+ *su-2*+)	1:3	.....
15–7 × NSa	Apparent wild type (*rg-1*+ *rg-2 su-2*)	× Apparent wild type (*rg-1*+ *rg-2 su-2*)	0:1	.....
B53 × NSa	*ragged* (*rg-1 rg-2*+ *su-2*+)	× Apparent wild type (*rg-1*+ *rg-2 su-2*)	..	*rg type-0*, *rg type-1*, *rg type-2*

The actual numbers of *rg* and wild-type progeny scored from the first three crosses, were 123 *rg* and 64 wt (B-1 × SFT-9); 104 *rg* and 56 wt (M-17 × NSa) and 153 *rg* and 77 wt (M-17 × 15-6). Out of 76 progeny examined from the cross 15-7 × 412, numbers of *rg* and wt were 20 and 56 respectively. On an average, 100 progeny were examined from each of the remaining crosses giving *rg*:wt = 3:1 or 1:1.

NSa and *rg type-2* (SFT-5) × AWT (15–6) also produced *rg* and wild-type progeny in the ratio of 1:1; however all the mutant progeny were *rg type-2*.

Both the cross *rg type-1* (M-17) × Whitehouse wild type and the cross *rg type-1* (M-17) × 412 gave *rg*:wt in the ratio of 1:1 both on random spore analysis and in asci. Among the mutant progeny from these crosses all were *rg type-1*. Also the crosses of *rg type-1* (SFT-1) × Whitehouse wild type and of *rg type-1* (SFT-1) × FGSC No. 412 gave *rg*:wt in the ratio of 1:1; all the *rg* progeny were *rg type-1*. The cross *rg type-2* (SFT-5) × Whitehouse wild type gave *rg* and wild-type progeny in the ratio of 1:1; all the *rg* progeny were *rg type-2*.

Crosses of *rg type-0* (M-13 or M-16) × Whitehouse wild type and *rg type-0* (M-13 or M-16) × FGSC No. 346, 414, 415, or 417 gave *rg* and wild-type progeny in the ratio of 3:1, *rg* progeny from these crosses included the three mutant phenotypes *rg type-0*, *rg type-1* and *rg type-2*. Also, the cross *rg type-1* (M-17) × *rg type-2* (SFT-2) yielded *rg* and wt progeny in the ratio of 3:1. The cross AWT (15–7) × wt (FGSC No. 412) produced one fourth *rg* progeny, while the crosses AWT (15–7) × NSa and AWT (15–7) × AWT (15–6) produced no *rg* progeny. The cross B53 (*rg*) × NSa was found to produce the three types of *rg* progeny (*rg type-0*, *rg type-1*, *rg type-2*).

DISCUSSION

Any explanation of the above results must account for the fact that the only *rg* marker introduced into *N. sitophila* was derived from the *N. crassa* strain B53, and it must also account for the presence of the *rg* isolates obtained in *N. sitophila* which differ phenotypically on the basis of recombination and complementation patterns. Clearly, additional mutant loci must be postulated. The simplest hypothesis requires the presence of two additional loci, one which may be described as *rg-2* and another as *su-2*; thus the latter is a suppressor of the mutant locus *rg-2*. In the terms of this hypothesis, the specific genotypes for the various strains described are given in Table 1.

The occurrence of the three *rg* phenotypes among the mutant progeny of the cross *rg type-0* × wild type suggests that *rg type-0* contains both the *rg-1* and *rg-2* loci. The fact that *rg type-1* (M-17) gave only one class of asci on crossing with the Whitehouse wild type and with FGSC No. 412, whereas it gave three classes of asci on crossing with NSa and AWT (15–6), suggests that NSa and AWT are genotypically different from other wild-type strains of *N. sitophila*. Thus it would appear that NSa and AWT are, in fact, suppressed *rg* having the genotype *rg-1*$^+$ *rg-2 su-2*. This is confirmed by the recovery of the *rg* progeny with different phenotypes from the cross *rg* B53 × NSa.

On the basis of the proposed hypothesis, crosses of *rg type-1* (M-17) × NSa, *rg type-1* (M-17) × 15–6 and *rg type-1* (B-1) × *rg type-2* (SFT-9) should all be heterozygous for the three pairs of alleles: *rg-1*/*rg-1*$^+$, *rg-2*/*rg-2*$^+$ and *su*-2/su-2$^+$. Proof of heterozygosity for these loci is provided by the recovery of the three types of asci (*rg*:wt = 4:4, *rg*:wt = 6.2, and *rg*:wt = 8:0) from each of these crosses. The complementation pattern of the *rg* progeny in three classes of asci

TABLE 3

Tetrad data for crosses showing different classes of asci in N. sitophila

Cross	Parent types and inferred genotypes	Number of asci in each ascus class			Total
		Class I (4*rg*:4wt)	Class II (8*rg*:0wt)	Class III (6*rg*:2wt)	
B–1 × SFT–9	*rg type-1* (*rg-1 rg-2+ su-2*) × *rg type-2* (*rg-1+ rg-2 su-2+*)	11	11	5	27
M–17 × NSa	*rg type-1* (*rg-1 rg-2+ su-2+*) × Apparent wild type (*rg-1+ rg-2 su-2+*)	11	7	4	22
M–17 × 15–6	*rg type-1* (*rg-1 rg-2+ su-2+*) × Apparent wild type (*rg-1+ rg-2 su-2*)	11	10	10	31
M–17 × Wa	*rg type-1* (*rg-1 rg-2+ su-2+*) × wild type (Whitehouse) (*rg-1+ rg-2+ su-2+*)	15	0	0	15
M–17 × 412	*rg type-1:* (*rg-1 rg-2+ su-2+*) × wild type (FGSC) (*rg-1+ rg-2+ su-2+*)	30	0	0	30
SFT–1 × NSa	*rg type-1* (*rg-1 rg-2+ su-2*) × wild type (*rg-1+ rg-2+ su-2+*)	20	0	0	20
SFT–1 × 15–6	*rg type-1* (*rg-1 rg-2+ su-2*) × Apparent wild type (*rg-1+ rg-2 su-2*)	20	0	0	20

occurs as predicted on the basis of the hypothesis. Data from these crosses, both from random spore analysis and from ascus analysis suggest that the three loci *rg-1*, *rg-2* and *su-2* are unlinked. Frequent occurrence of tetratype asci with the three *rg* phenotypes and the wild-type progeny in each ascus indicates that both *rg-2* and *su-2* are some distance away from their respective centromeres. Further evidence for the present hypothesis is derived from the recovery of *rg* progeny from the cross AWT × wt.

The fact that isolate SFT-1 (*rg type-1*) yielded *rg* and wt progeny in the ratio of 1:1 in crosses with NSa, with AWT and also with the Whitehouse wild-types strains is explained on the basis of the isolate SFT-1 itself containing the suppressor gene *su-2*. That *rg type-1* isolates may or may not contain the suppressor gene *su-2* clearly shows that the latter has no effect on *rg-1*. Also, the occurrence of asci with 4 *rg type-1*:4 wt in the cross of *rg type-1* (B-1) × *rg type-2* (SFT-9) reveals the specificity of the suppressor gene, *su-2*, for the *rg-2* locus. This may also be inferred from various other crosses.

The present data indicate that in *N. sitophila*, all wild-type strains except the apparent wild type, which were recovered from the *rg* × *rg* cross, and NSa, are in fact *rg-1*$^+$ *rg-2*$^+$. It is also inferred that these wild-type strains, except the NSa and AWT do not contain the mutant suppressor gene *su-2*. If the *su-2* gene was present in the wild-type strain (Wa) then the cross wild type (Wa) × *rg type-2* should have yielded *rg* and wt progeny in the ratio of 1:3 instead of 1:1 as observed (see Table 2).

It is of interest to consider the origin of the postulated *rg-2* and *su-2* loci. It is possible that the *su-2* locus may have been present in *N. crassa* wild-type strains and that the mutation *rg-2* may have occurred during the investigation. Alternatively both mutations may have occurred during the investigation. A further possibility is that some *N. crassa* wild-type strains may contain both *rg-2* and *su-2* alleles. Data from the cross of B53 × NSa, where both *rg type-2* and *rg type-0* have been recovered, suggest that this cross must be heterozygous for both *rg-2* and *su-2*. Data from crosses of B53 in *N. crassa* (PERKINS 1959) show that the progeny of *rg*:wt occur in a 1:1 ratio. The combined information from these crosses with strains B53 points to the fact that B53 must be of the genotype *rg-1* *rg-2*$^+$ *su-2*$^+$ while the *N. crassa* wild-type strains must have the genotype *rg-1*$^+$ *rg-2*$^+$ *su-2*$^+$. Thus the second possibility described above appears to be likely, i.e. both mutations occurred during the course of the investigation. However, the possibility of new combinations of alleles brought about through the hybridization of *N. crassa* with *N. sitophila*, giving rise to what appear to be new mutations can not be ruled out. Thus it is possible that the *su-2* effect may be the result of a rare recombination between two closely linked alleles (one derived from *N. crassa* and the other from *N. sitophila*) neither of which would behave as a suppressor when present separately. A further possibility is that *N. crassa* alleles may remain undetected in their own genome but may be revealed when transferred to the genome of *N. sitophila*.

The mechanism of suppression of *rg-2* by *su-2* is not yet known but is under investigation.

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SUMMARY

During the transfer of the *rg* (ragged) markers from *N. crassa* to *N. sitophila* a new morphological mutant was detected, which has been designated *rg-2* by virtue of its phenotypic similarity to the known *rg* mutant in *N. crassa*, (now numbered *rg-1*). A further mutant capable of suppressing *rg-2* in *N. sitophila* has also been detected, and designated *su-2*. The three markers *rg-1*, *rg-2* and *su-2* are unlinked and in various combinations they give rise to a number of genotypes associated with the *rg* phenotype, and also to an apparent wild type with the genotype *rg-1*$^+$ *rg-2 su-2*. The origin of these newly detected loci is discussed.

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